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Research Article

Assessing the upper thermal limit constraining the physiological performance of *Callinectes sapidus* embryogenesis under climate warming scenarios

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Abstract

Embryonic development represents a vulnerable life stage in marine organisms, yet its role in shaping the invasion success of non-native species under climate change remains understudied. In this study, we assessed the upper thermal sensitivity of embryogenesis of the blue crab *Callinectes sapidus*, a globally invasive species, by quantifying their physiological responses across a temperature gradient relevant to projected climate warming scenarios. Using Electron Transport System (ETS) activity as a proxy for aerobic metabolism, we evaluated respiration, egg size, hatching time, and larval morphology in brooding eggs incubated at 22 °C, 24 °C, 26 °C, and 28 °C. Elevated temperatures induced increased ETS activity, indicating heightened metabolic stress, and were associated with reduced egg size and earlier hatching of malformed, non-viable larvae. Within the Oxygen- and Capacity-Limited Thermal Tolerance (OCLTT) framework, we identified a physiological pejus range (24–26 °C) beyond which embryonic performance declined. These results suggest that moderate warming may accelerate development and facilitate invasion, but extreme temperatures constrain aerobic capacity and compromise larval viability. Our results highlight embryogenesis as a potential bottleneck for blue crab recruitment under future warming, with implications for predicting the invasive potential of marine species.

Key words: *Callinectes sapidus*, ecophysiology, ETS, heatwaves, larval ecology, maternal effects, thermal stress

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Introduction

Phenological windows have been suggested as a part of a warning system enabling more targeted programs for monitoring invasive species (Giménez et al. 2020). Warming coastal waters have likely contributed to the recruitment and northward expansion of the invasive blue crab *Callinectes sapidus* Rathbun, 1896, beyond its historical range at Cape Cod, resulting in the establishment of permanent populations in new areas (Johnson 2015; Taylor et al. 2022; Crane et al. 2024). The American blue crab *C. sapidus* (Brachyura, Portunidae) is native to the Atlantic

coast, ranging from Nova Scotia, Canada, to northern Argentina, including the Gulf of Mexico (Squires 1990). Outside its original distribution, the species has established invasive populations in various regions of Africa, Asia and Europe (Nehring 2011). In the Iberian Peninsula, the first record of the species occurred in the Tagus Estuary in 1978 (Gaudencio and Guerra 1979). Since then, it has rapidly spread along the Spanish coasts, reaching the Guadalquivir Estuary in the Gulf of Cadiz by around 2017 (González-Ortegón et al. 2020).

The primary vector for the introduction of *C. sapidus* is thought to be ballast water releases, which can transport planktonic larvae during uptake (Nehring 2011). Considering that larval development in *C. sapidus* lasts 37–69 days, the timeframe allows for plausible transoceanic transport, although secondary dispersal is also possible (Nehring 2011). The species' successful establishment can be attributed to several ecophysiological and developmental traits, such as the larvae's high resistance to abiotic and biotic conditions (Anger 2006; Morais et al. 2019), enabling adaptation to a wide range of environmental factors.

The larval ecology of *C. sapidus* is of particular interest, as adult population recruitment depends on the survival of larvae and juveniles to replenish the parental stock (Sandifer 1975), acting like a population bottleneck. Larval survival is a vulnerable life stage heavily influenced by environmental factors, particularly seawater temperature and salinity (Costlow and Bookhout 1959; Costlow 1967; Rosenberg and Costlow 1976; Rumrill 1990; Anger 2006). While numerous studies have been conducted on the biology and ecology of *C. sapidus* in its native range, most research has focused on its commercial importance and the environmental conditions in its original habitats (Olmi and Orth 1995; Daly et al. 2021). However, there is a notable lack of studies addressing the effects of environmental changes in invaded regions such as the Gulf of Cadiz.

The Gulf of Cadiz is a temperate and warm ecosystem enriched by river discharges that inject nutrients and trace metals which stimulates primary and secondary production (Prieto et al. 2009; González-Ortegón et al. 2019), thus creating a potentially suitable environment for the development of invasive species like *C. sapidus*. The Gulf of Cadiz connected to the Mediterranean Sea through the Strait of Gibraltar, facilitates a two-layer water exchange: a surface inflow from the Eastern North Atlantic and deeper outflow of Mediterranean saline waters (Sánchez-Leal et al. 2017). This hydrodynamic regime, along with significant anthropogenic transformation along the coast, including major ports like Algeciras and extensive aquaculture infrastructure (González-Ortegón and Moreno-Andrés 2021), may have facilitated the westward expansion of the invasive Atlantic blue crab *Callinectes sapidus* from the Mediterranean into the Atlantic. Genetic evidence supports a single invasion event into the Mediterranean followed by secondary spread into adjacent Atlantic waters (González-Ortegón et al. 2022). The potentially less extreme upper thermal limits in the Atlantic region may offer more favourable conditions for the species' establishment and survival. This is consistent with the invasive success of *C. sapidus*, which generally exhibits wider thermal tolerance and may possess adaptive advantages over native species such as *Carcinus maenas* (Anger 2006; Nehring 2011).

Laboratory experiments suggested that *C. sapidus* requires high temperatures (above 21 °C) for optimal larval development (Hill et al. 1989; Bembe et al. 2017). Combined with the warm sea temperatures of the Gulf of Cadiz, this raises the question of how these conditions will affect its development and expansion in the

region. While most studies focus on estimates of critical thermal maxima (CT_{max}) or lethal limits (LT_{50}) when exposed to thermal stress, insights on the intermediate physiological constraints are more relevant in ecological studies, especially in organisms with critical life stages as crustaceans. Exploring the animal Oxygen- and Capacity- Limited Thermal Tolerance (OCLTT) through their physiological performance, could provide valuable information about their climate responses in a more realistic scenario of gradual rising temperatures (Pörtner et al. 2017).

In this sense, the objective of the present study is to assess the upper thermal limit constraining the embryogenesis performance of *C. sapidus*, as the global warming context in the Gulf of Cadiz could act as a bottleneck to population recruitment. Performance is explored through interlinked parameters characterising embryonic thermal sensitivity and aerobic window, as the Electron Transport System (ETS) activity, egg size development, hatching success, and larval morphology. ETS activity assays, a potential indicator of respiration, is a common proxy for planktonic respiration (Packard 1971; Herrera et al. 2017), and has proven effective for estimating metabolic rates in crustaceans (Simčič and Brancelj 2004; Simčič et al. 2014; Ruiz-Delgado et al. 2019; Herrera et al. 2024). Exploring ETS activity on brooding eggs of *C. sapidus* exposed to different temperatures could provide insights into variations on the metabolic dynamics during embryonic development under warming conditions. Egg size dynamics over incubation time may be an indicator of development rates, which coupled with hatching time and larval morphology, provides valuable information about larval fitness and survival success under warming conditions.

Methods

Egg collection and experimental design

Eggs masses of *Callinectes sapidus* were reared in the laboratory to assess the effects of increased seawater temperature on embryonic development prior to larval hatching. Eggs were obtained from six ovigerous females (referred as F1, F2, F3, F4, F5 and F6) at similar stages of embryonic development. These ovigerous females were manually collected by a local fisherman in a shallow inlet of the Guadalquivir Estuary (Sanlucar de Barrameda, Gulf of Cadiz, Spain) during August 2023. Genetic analysis was performed to determine the haplotype of each female, in order to explore whether the existence of possible outliers in the statistical analysis could be attributed to genetic differences between them. Two different haplotypes of *C. sapidus* coexist in the studied area: CSWM1, predominant in Spanish Atlantic coast; and CSWM2, predominant in Spanish Mediterranean coast (González-Ortegón et al. 2022). In our study, F1, F2, F3, F5 and F6 belonged to CSWM1 haplotype, while F4 belonged to CSWM2.

Each egg mass was removed from the female abdomen and its volume was evenly divided among four temperature treatments in individual containers (1000 ml): 22 °C, 24 °C (defined as control), 26 °C, and 28 °C. These temperatures were selected based on the (i) available bibliography which settle the lower thermal limit for the successful embryonic development (> 21 °C) (Jivoff et al. 2007; Bembe et al. 2017), (ii) the mean summer water temperature at the sampling site of ovigerous females when brooding and spawning season occur (24 °C), and (iii) the IPCC RCP8.5 projected warming scenarios of +2 °C and

+4 °C for the end-of-century (Pörtner et al. 2019). One group was kept at a controlled temperature, while the others were gradually exposed to different experimental temperatures (22 °C, 26 °C, and 28 °C). After 24 hours at 24 °C (T0), they were gradually transferred to 22 °C and 26 °C (T1) over a 24-hour period, while another group, previously at 26 °C, was transferred to 28 °C one day later over a 24-hour period (T4), allowing the eggs time to adapt to the three new temperatures (Fig. 1). Filtered seawater (35 of salinity) was renewed daily, to prevent fungal, bacteria and other microorganism's growth in the egg mass.

Embryonic development

During the experiment, a sample of eggs per treatment and female was collected for embryonic developmental analysis, in order to test the possible effects of temperature on egg size over time. Samples were stored in 2 mL Eppendorf tubes containing seawater at -20 °C. For each sample, the major diameters of twenty randomly selected eggs were measured under a stereo microscope (SMZ25/18, Nikon Instruments Inc.) using the NIS-elements Imaging Software v. 5.21.00. Thus, the median egg size (μm) and standard deviation were calculated. Images were captured at a scale of 250 μm.

Electron Transport System (ETS) assay

Egg samples (~15 mg) from each treatment and female were collected daily to measure respiratory ETS activity (in μL $O_2 \cdot h^{-1} \cdot$ mg $prot^{-1}$), reflecting cellular-level changes during embryonic development. Samples were preserved in Eppendorf tubes, frozen in liquid nitrogen (-196 °C), and stored at -80 °C prior to analysis. The ETS assay followed the method of Packard (1971), modified by Owens and King (1975), and adapted for microplate readings by Ruiz-Delgado et al. (2019). Eggs were homogenized in 0.5 mL of ice-cold homogenizing buffer solution (20 mM Trizma base, pH 7.8, Sigma-Aldrich) using an ultrasonic homogenizer (UP2005 Hielscher) set to 1 cycle at 25% amplitude for 60 seconds. The homogenate was centrifuged at 3 °C for 10 minutes at 5,000 rpm (Eppendorf Centrifuge 5417R), and the supernatant was used for the ETS assay. In a microplate assay, 60 μL of supernatant (in duplicate) was incubated with 180 μL of substrate solution (0.1 M phosphate buffer, pH 8.5, containing NADPH 30 mM and NADH 1.76 mM, Sigma-Aldrich) or without substrate (control, containing only 180 μL of phosphate buffer, pH 8.5). Then, 60 μL of INT solution (0.2%, 4 mM, pH 8.5) was added to each sample, and absorbance was measured at 490 nm over 8 minutes using a microplate reader (BioTek Synergy H1) and Gen5 3.10 software. ETS activity was corrected for *in situ* temperature using an activation energy of 15 kcal·mol^{-1} (Packard 1971) and the Arrhenius equation to calculate *in situ* ETS activity (units: μL $O_2 \cdot h^{-1}$). To calculate ETS activity per unit biomass, protein biomass (mg protein) was determined using the bicinchoninic acid (BCA) method described by Smith et al. (1985). For this, 25 μL of each sample and standards were incubated with 200 μL of BCA working solution for 30 minutes at 37 °C, and absorbance was read kinetically at 562 nm. ETS activity was then normalized to protein content and expressed as ETS activity per unit protein (μL $O_2 \cdot h^{-1} \cdot$ mg $prot^{-1}$).

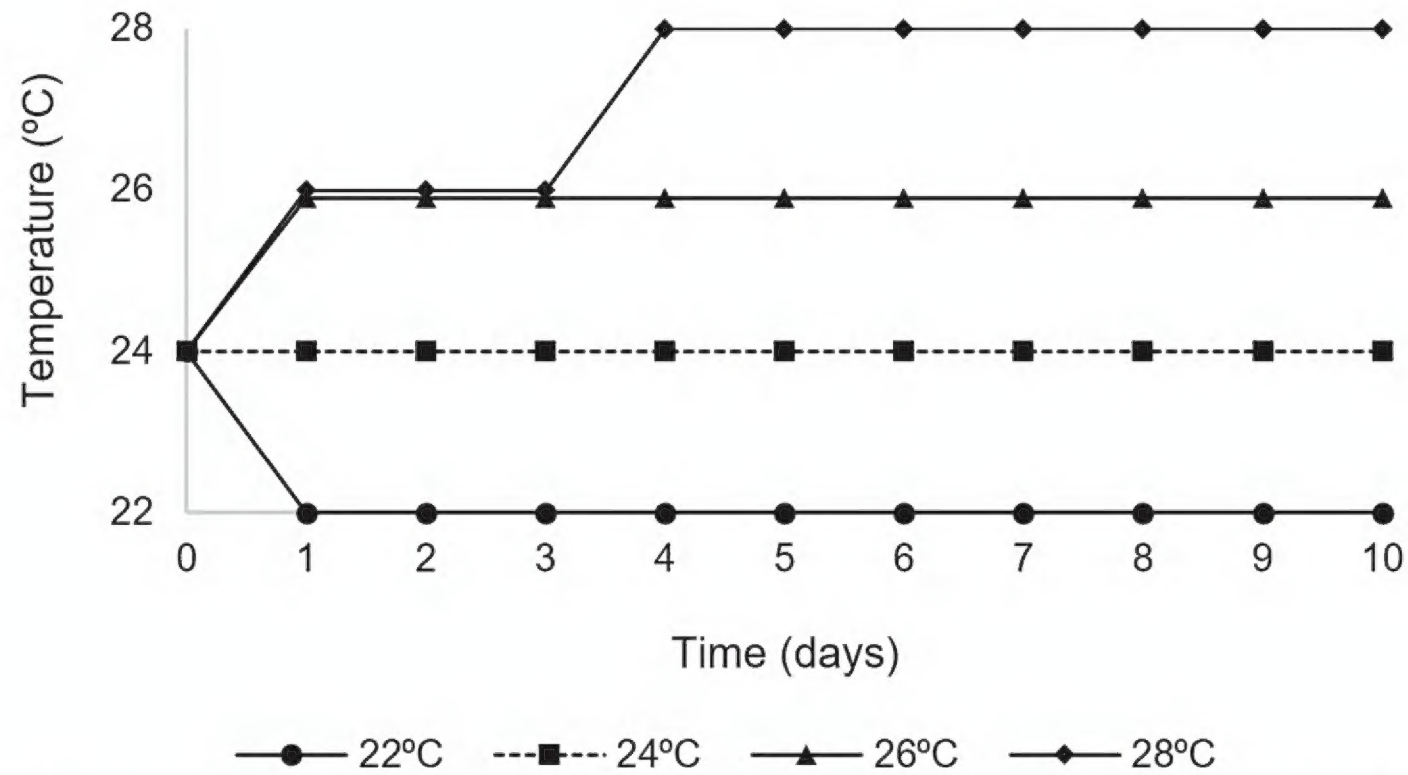

Figure 1. Experimental design. Schematic diagram of the temperature treatment design and acclimation protocol applied to *C. sapidus* embryos across the experimental period (T0-T10). Dashed line indicates control at 24 °C.

Egg hatching time and larval viability

Egg mass cultures were maintained under different temperature treatments until larval hatching, which marked the end of the experiment for each sample condition. Hatching time was recorded as the number of days from day 0 (the start of incubation) until larvae appeared.

The approximate number of newly hatched larvae was counted for each treatment and female. Observations on the morphological condition and motility of the larvae were documented to assess their viability. This included noting the presence or absence of aberrant zoea, and evaluating their phototactic swimming behavior, according to Jivoff et al. (2007). In this study, aberrant zoea is defined as an individual at a morphological stage similar to prezoea but that is immobile, exhibits sinking behaviour, and subsequently dies. In contrast, under optimal conditions, prezoea molts into the first zoeal stage within the first 3 minutes of life after their release (Costlow and Bookhout 1959; Davis 1965).

Statistical analysis

The relationship between predictors with the ETS activity ($\mu L\ O_2 \cdot h^{-1} \cdot mg\ prot^{-1}$) and egg size (μm) of *C. sapidus* embryos was examined through a Generalized Additive Model (GAM) with lognormal and normal distribution, respectively, based on the distribution of the dependent variables (Zuur et al. 2009). The GAM model was selected as it assumes no functional form between dependent and independent variables and describes both linear and non-linear effects, and was performed on RStudio software v.2024.12.0. A variance inflation factor (VIF) with a threshold of 3 was used to identify possible collinearity between predictors in the data set, before fitting models to the data (Zuur et al. 2010). This analysis indicated no multicollinearity as all explanatory variables had VIF < 3 (see Suppl. material 1: tables S1, S2), so the general form of the GAM was:

$$g(y) \sim \alpha + f_1(x_i) + \varepsilon_t + \varepsilon_f$$

where *g()* is the link function, *y* is the response variable (ETS activity or egg size), f_1 is the smooth function for the x_i continuous explanatory variable (incubation time), ε_t is the categorical effect of the temperature treatments, and ε_f is the random effect of female origin of embryos, capturing its intrinsic variability. Also, due to the unbalance representation of haplotypes, this factor was not included in the GAM analysis. Estimated R^2 and explained deviance were used to evaluate the predictive performance of the model. The residuals were graphically evaluated with QQ-plots, histograms, and plots of residual response against fitted values to explore any patterns in the residual errors (see Suppl. material 1: figs S2, S3).

Post-hoc analysis using the Tukey test for varying family sizes was performed for identifying significant differences between specific levels of the categorical factor affecting embryo ETS activity and egg size.

Results

Temperature-dependent variation in egg size

A general pattern of decreasing egg size over development time was observed across all temperature treatments (see Suppl. material 1: fig. S1). Under control conditions (24 °C), among all females, eggs at the very early stage of embryonic development had a mean diameter of 273.4 ± 13.8 µm, which decreased to 241.5 ± 12.8 µm just before hatching, a reduction of approximately 11.7%.

The global median egg size resulted in 247.59 µm. Smaller egg sizes, below 245 µm, were found under warmer conditions of 26 °C and 28 °C; while larger eggs, above 245 µm, resulted in colder temperatures of 22 °C and 24 °C, reaching the maximum at control conditions of 24 °C (306.40 µm) (Table 1). These results suggest that elevated temperatures negatively affect embryonic development by reducing egg size.

GAM analysis showed significant effect of incubation time, temperature treatment, and maternal origin on egg size (see Suppl. material 1: table S1). The model explained a moderate portion of the variability in egg size (R^2 = 0.282; deviance explained = 28.7%), with all predictors showing significant effects ($p < 0.001$). The effect of incubation time on egg size was non-linear and highly significant (*edf* = 7.37, $F = 70.02$, $p < 0.001$). The smooth function in Fig. 2A indicated a sharp decline in egg size during the early stages of embryo development, followed by a period of stabilization and a secondary decline around T8. Confidence intervals around the smooth term suggested a precise estimation of this temporal pattern, with wider intervals in later stages, where data density is lower due to different larvae hatching times. The random effect of maternal origin was significant as well (*edf* = 4.78, $F = 20.71$, $p < 0.001$), indicating that individual females contributed to variability in egg size, justifying its inclusion as a random smoother in the model. Although the genetic background of females could be a factor contributing to the variability in egg size, the presence of outliers was not restricted to a single female (F4 belonged to CSWM2 haplotype), but rather distributed across several individuals.

Temperature treatments also significantly affected egg size ($F = 39.51$, $p < 0.001$). Compared to the reference level (22 °C), egg size decreased significantly at higher temperatures of 26 °C and 28 °C (estimates of -6.77 µm and -4.17 µm, respectively; $p < 0.0001$ for both cases), and to a lesser extent at 24 °C (Estimate = -1.73 µm; $p = 0.016$). The Tukey-adjusted post hoc comparisons confirmed that all pairwise differences were significant, except between 22 °C and 24 °C temperature groups

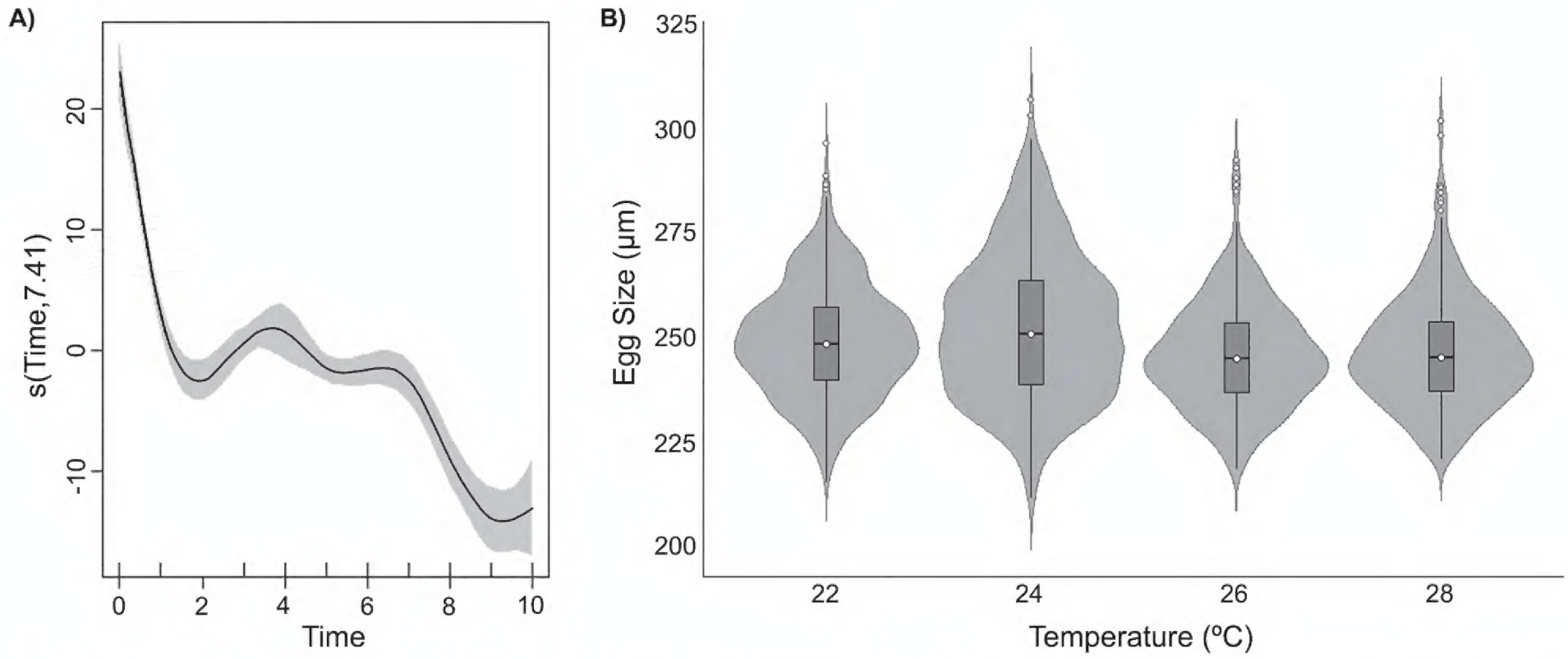

Figure 2. Graphical representation of the effects of the incubation time and temperature on *C. sapidus* egg size (µm). Results of the GAM analysis showing **A.** Partial effect of incubation time (during 10 days) on egg size, where the black solid line indicates the modelled relationship, and the grey band denotes the 95% confidence interval about the estimated relationship, and **B.** Violin plot showing the distribution of egg size under tested temperature treatments (22 °C, 24 °C, 26 °C, and 28 °C), where boxplots embedded within the violins indicate the interquartile ranges and central tendency.

Table 1. Egg size (µm) summary by temperature treatment. Median egg size (µm), standard deviation (SD), and range (maximum and minimum values) per temperature treatment.

Temperature (°C)	Median ± SD (µm)	Maximum (µm)	Minimum (µm)
22	248.22 ± 13.21	296.06	215.03
24	250.55 ± 17.34	306.40	211.15
26	244.49 ± 12.64	291.90	218.21
28	244.84 ± 13.24	301.26	220.66

(see Suppl. material 1: table S1). These results indicated that small increments in temperature beyond the optimal range can influence egg development. The violin plot in Fig. 2B supported these findings, providing a visual summary of both central tendency and data distribution. The boxplots nested within the violins highlight the central tendency of decreasing median egg size with increasing temperature, as well as increasing outlier prevalence at 26 °C and 28 °C, suggesting a stress-related developmental constraint.

Electron Transport System (ETS) activity assay

Embryos ETS activity, a proxy for potential respiration rates and expressed as log-transformed specific ETS activity, was significantly influenced by incubation time, temperature treatment, and maternal origin (see Suppl. material 1: table S2). The GAM explained 61.4% of the deviance in ETS activity (R^2 = 0.584), indicating strong explanatory power of those selected variables and reinforcing the biological relevance of thermal and maternal influences on embryonic metabolic performance. The smooth effect of incubation time was significant (*edf* = 2.80, *F* = 33.14, $p < 0.001$), indicating a non-linear increase in ETS activity throughout the incubation period (Fig. 3A). The fitted smooth term suggested a gradual acceleration of ETS activities over time, with a pronounced increase around T4 (4th day), when

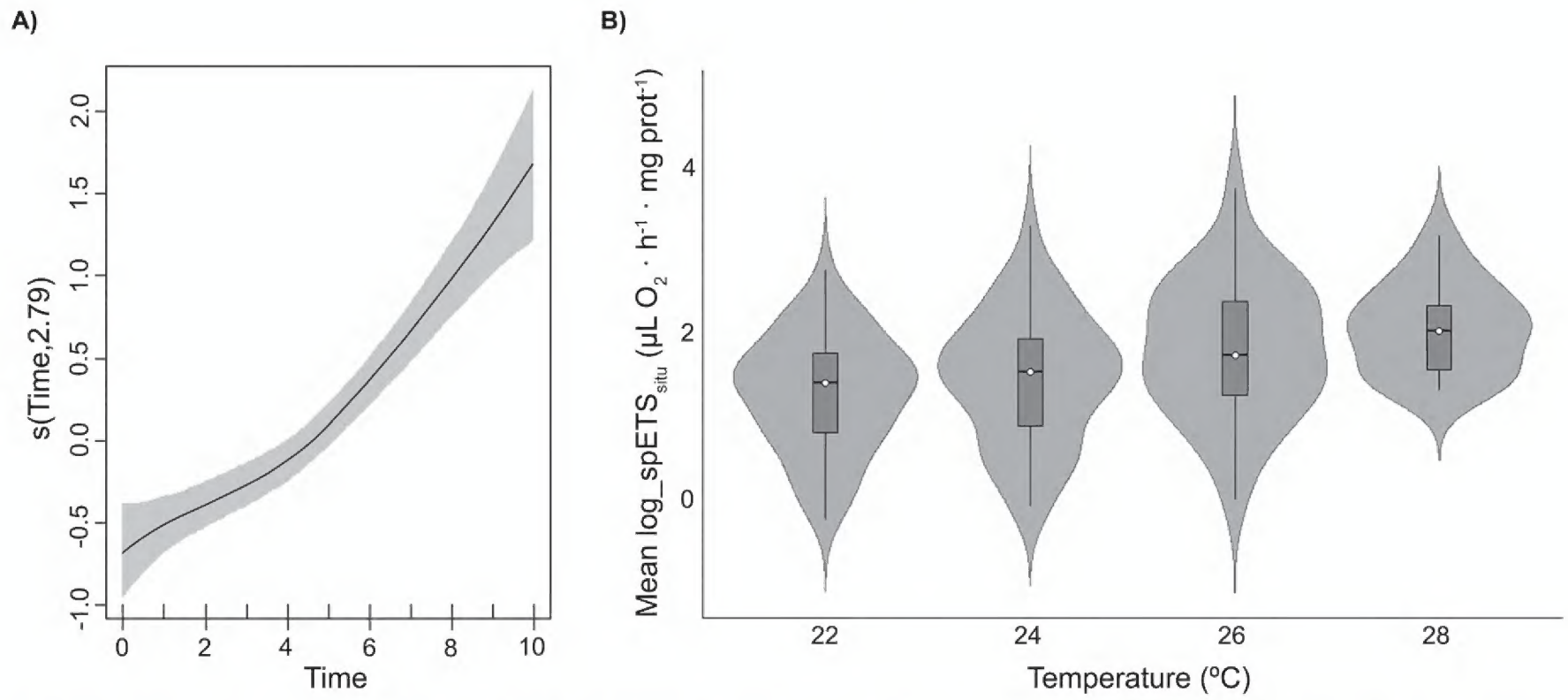

Figure 3. Graphical representation of the effects of the incubation time and temperature on ETS activity (µL O2 · h–1 · mg prot–1) of *C. sapidus* embryos. Results of the GAM analysis showing **A.** Partial effect of incubation time (during 10 days) on ETS activity, where the black solid line indicates the modelled relationship, and the grey band denotes the 95% confidence interval about the estimated relationship, and **B.** Violin plot showing the distribution of ETS activity under tested temperature treatments (22 °C, 24 °C, 26 °C, and 28 °C), where boxplots embedded within the violins indicate the interquartile ranges and central tendency.

the experimental increase in temperature to 28 °C was performed. This point of inflection indicates a metabolic shift, followed by continued increasing of ETS and widening confidence intervals toward the later stages of development. The effect of maternal origin was also highly significant (*edf* = 4.75, $F = 17.31$, $p < 0.001$), underlining individual female variability in the baseline metabolic activity during embryogenesis. Although the genetic background of females could be a factor contributing to the variability in ETS activity, the presence of outliers was not restricted to a single female (F4), but rather distributed across several individuals.

Temperature treatments strongly affected ETS activity of embryos. Compared to the reference level of 22 °C, ETS activities were significantly higher at major temperature regimes of 24 °C (Estimate = 0.39, $p < 0.001$), 26 °C (Estimate = 0.82, $p < 0.0001$), and 28 °C (Estimate = 0.79, $p < 0.0001$). Post hoc Tukey-adjusted comparisons confirmed significant pairwise differences between 22 °C and all other treatments, as well as between 24 °C and both 26 °C and 28 °C (see Suppl. material 1: table S2). ETS activity seemed to become constant at the two highest temperatures, with no significant difference between 26 °C and 28 °C ($p = 0.998$), suggesting a threshold effect where ETS activity ceases to increase despite additional warming. The Fig. 3B corroborated this pattern with wider distributions and higher medians under warmer conditions, consistent with thermally enhanced metabolic rates. The shape of the violins indicates relatively symmetric distributions, contrasting with the right-skew observed in egg size.

Hatching time

A general pattern of early larval hatching at higher temperatures was observed (Table 2), which was also confirmed by the photographic analysis of the morphological characteristics on egg development samples (Fig. 4). In all females, early hatching occurred between 6–7 days at the highest temperatures (26–28 °C), while hatching at lower temperatures (22–24 °C) was delayed, taking up to a maximum of 11 days.

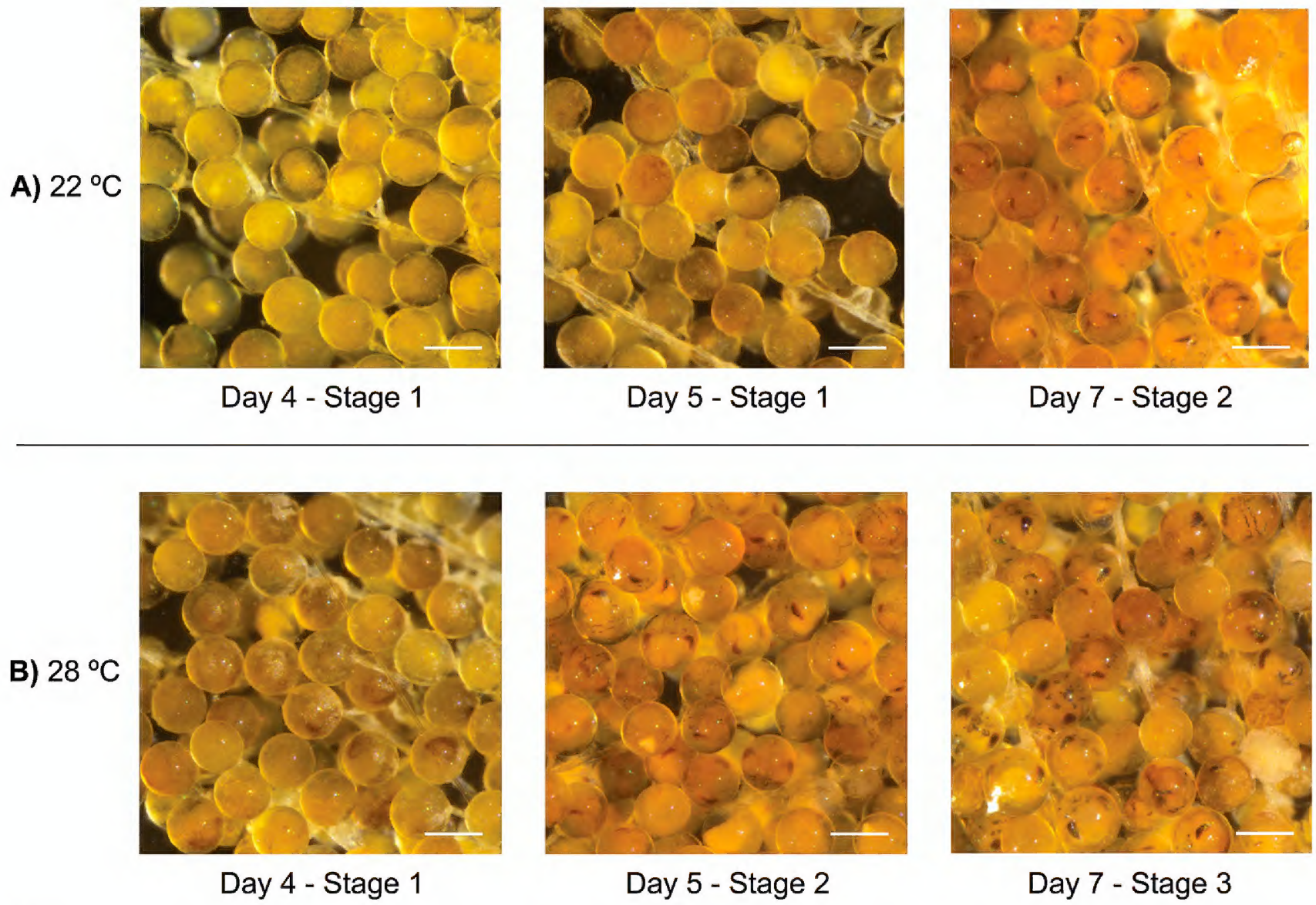

Figure 4. Comparative morphological analysis of eggs' development between temperature treatments. Embryonic development of blue crab eggs from female F4 at two different temperatures **A.** 22 °C, and **B.** 28 °C, over three different times: 4-day-old embryos, 5-day-old embryos, and 7-day-old embryos. At stage 1, egg attachment stalks are visible; at stage 2, eye pigment is developing; and at stage 3, abdomen and fully formed eyes are visible, embryos are ready to hatch (Jivoff et al. 2007). Scale bar: 250 µm.

Table 2. Hatching time. Eggs hatching day per female and temperature treatment. The plus symbol in brackets indicates samplings where massive larvae hatching occurred (> 50 larvae hatched of total egg volume, similar across all temperature treatments and females). The asterisk symbol indicates the presence of aberrant larvae.

Female	Temperature (°C)			
	22	24	26	28
1	8 (+)	7 (+)	6*	6*
2	7 (+)	6	6*	6*
3	7	6	6*	6*
4	11 (+)	10 (+)	7*	0
5	11 (+)	10 (+)	7 (+)	7 (+)*
6	9 (+)	8 (+)	6*	7 (+)*

In general, lower temperatures resulted in a higher number of hatched larvae, although there were differences among females. Only a small number of larvae from females F2 and F3 hatched at lower temperatures (22–24 °C), but these larvae exhibited active and phototactic swimming behaviour, similar to those from other females under the same temperature treatments. In contrast, at 26–28 °C, early hatching often produced > 50 larvae initially, but many were aberrant or non-viable. Thus, thermal stress likely induced premature hatching, leading to malformations in larvae morphology (Fig. 5).

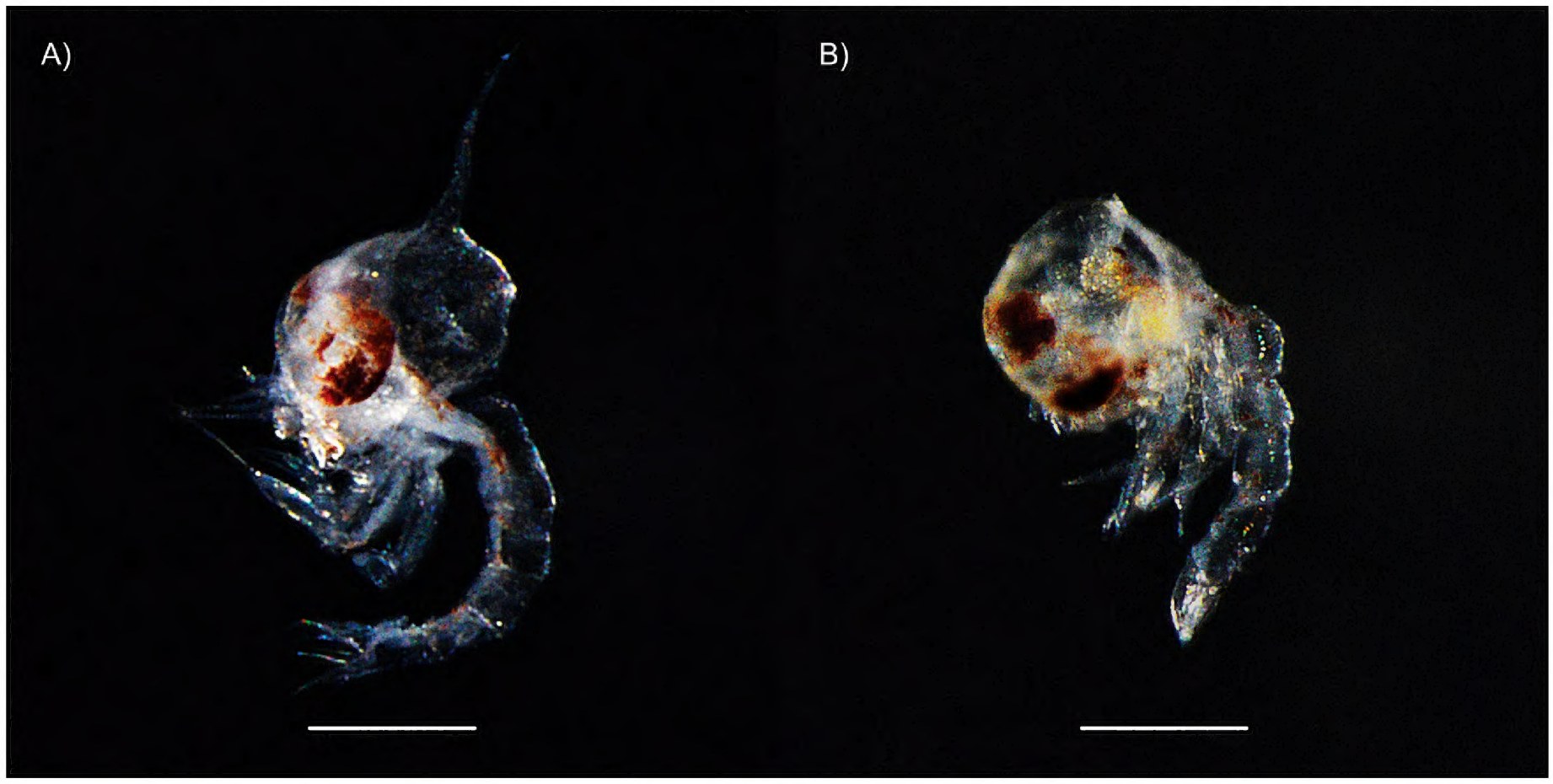

Figure 5. Comparative morphology between newly hatched larvae from different temperature treatments. Comparison between **A.** Newly hatched larva of *C. sapidus* from female 1 under control conditions at 24 °C, and **B.** Aberrant newly hatched larva from the same female at 28 °C treatment. Hatching day corresponding to T8 and T6, respectively. Morphological abnormalities observed in aberrant larva include the absence of the erect carapace spine, a fully formed telson, and appendages. Scale bars: 250 μm.

Discussion

This study provides a comprehensive analysis of the upper thermal limits in *C. sapidus* embryogenesis, under warming conditions in the Gulf of Cadiz, using interlinked physiological and developmental parameters. Egg size, metabolic activity (ETS), hatching time, and larval morphology were all significantly influenced by temperature treatments, collectively highlighting the constraints of elevated seawater temperatures on early life stages. Based on the results of this study, a schematic model showing the thermal window dynamics of *Callinectes sapidus* embryonic development is proposed (Fig. 6).

Egg size, a key proxy for development progress, decreased significantly at higher incubation temperatures of 26 °C and 28 °C, consistent with previous research (Jivoff et al. 2007; Epifanio 2019). This reduction in egg size, particularly evident at later developmental stages and higher temperatures, may reflect a temperature-induced acceleration of embryogenesis and increased yolk consumption per day, potentially at the cost of somatic growth and compromising hatching success. Similar trends have been reported in other brachyuran species, including *C. sapidus* (Amsler and George 1984; Jacobs et al. 2003; Graham et al. 2012; Styf et al. 2013). However, a general trend of decreasing egg size over incubation time was observed across all treatments, in contrast to previous studies on *C. sapidus* (Graham et al. 2012). This discrepancy highlights the variability of egg size in crustaceans, which could be influenced by other factors not included in the analysis, such as spawning season, maternal parity, and environmental conditions, especially seawater salinity (Davis 1965; Kobayashi and Matsuura 1995; Brante et al. 2003).

Embryonic ETS activity, used as a proxy for potential aerobic respiration, showed a temperature-dependent non-linear increase throughout development time, with pronounced elevations at higher temperatures (26–28 °C), reflecting embryonic thermal stress and accelerated metabolism rates (Herrera et al. 2017, 2019). These suboptimal conditions were also evidenced by the high variability in ETS responses, with wider data

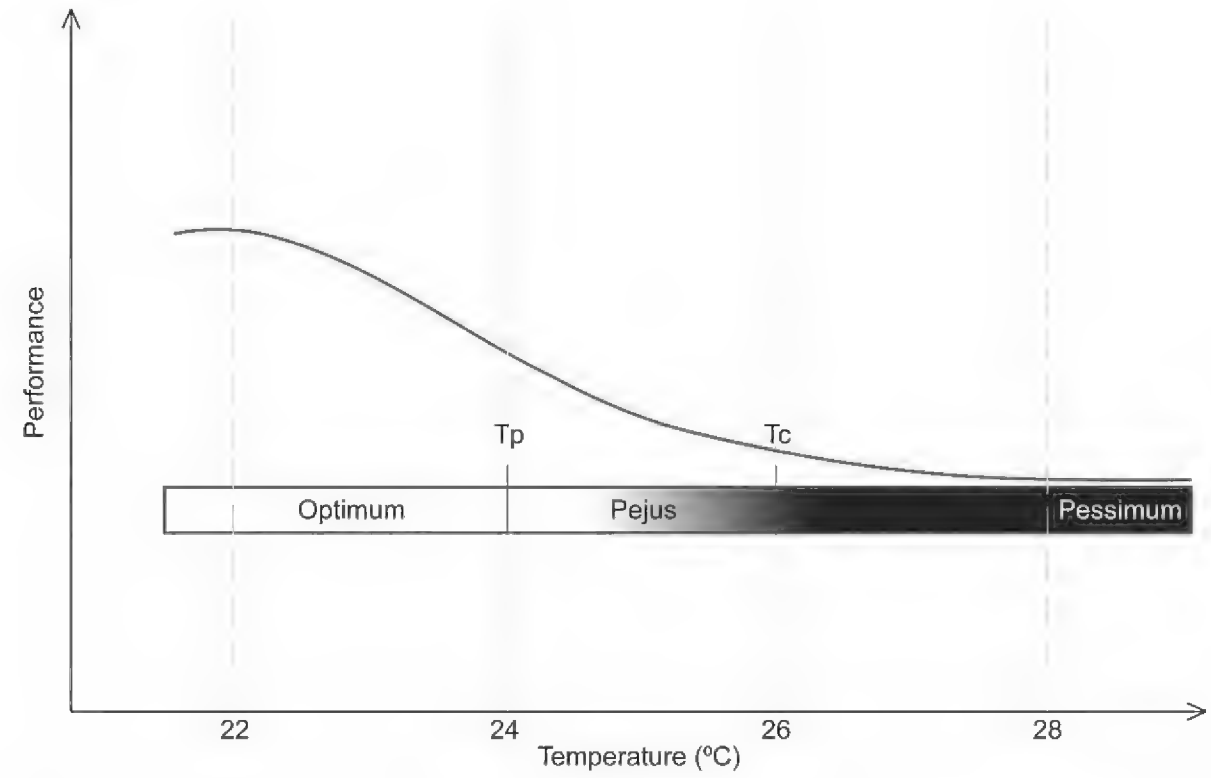

Figure 6. Schematic model for the thermal window dynamics of *C. sapidus* embryonic development. The interlinked phenomena characterizing development performance of egg size, ETS activity, hatching time, and larval morphology is represented against temperature treatments, graphically supporting the existence of an upper thermal limit for successful embryogenesis and larval fitness. Pejus temperature (T_p = 24 °C) highlight the temperature threshold in which a gradual decrease of performance occurs in the pejus range, until reaching the critical temperature (T_c > 26 °C).

distribution at extreme temperatures. In contrast, ETS activity increased more gradually and consistently at lower temperatures (22–24 °C), indicative of stable metabolic performance and better embryonic condition. This pattern aligns with the OCLTT framework (Pörtner et al. 2017), which proposes that aerobic scope narrows beyond optimal temperatures due to a mismatch between oxygen demand and delivery capacity. In this context, the observed peak and variability in ETS at elevated temperatures could indicate the onset of pejus conditions, where metabolic performance declines prior to lethality. The steady state reached in ETS levels between 26 °C and 28 °C, despite continued temperature rise, suggests the approach to a physiological threshold beyond which metabolic performance can no longer be upregulated. This supports the concept of an upper thermal limit, where additional warming fails to produce further metabolic gains and may instead incur developmental costs.

The observed trend of increasing embryonic ETS activity prior to larval hatching, likely correlates with the mechanical action by the larva's abdomen and telson. This behaviour enables the rupture of the outer membrane and larval emergence, a process requiring significant metabolic energy (Davis 1965). Hatching dynamics and larval morphology provided further evidence of this thermal stress. ETS activity in embryos under optimal temperatures (22–24 °C) resulted in higher hatching success and viable larvae, indicating that optimal development likely occurs within a narrower thermal window (Costlow and Bookhout 1959; Hill et al. 1989; Jivoff et al. 2007; Bembe et al. 2017). In contrast, larvae exposed to higher temperatures (26–28 °C) hatched earlier, often within 6 to 7 days, as seen in previous studies (Zheng and Kruse 2000; Orensanz et al. 2004; Marochi et al. 2021). Premature hatching before fully embryonic maturation, often resulted in fewer viable larvae. These larvae exhibited aberrant forms (prezoea stage), indicative of poor physiological condition. These morphological alterations included the absence of an erect rostral spine, incomplete formation of the telson, and missing appendages, features that distinguish them from the fully developed Zoea I stage, as described by Costlow and Bookhout (1959).

Aberrant larvae were immobile, exhibited sinking behaviour, and subsequently died, likely due to their inability to maintain active vertical swimming for feeding (Foxon 1934; Sulkin 1984; Young 1995; Sulkin et al. 2000).

The significant effect of maternal origin further supports the influence of intrinsic variability in shaping embryonic responses, as there could be several factors affecting female fecundity, including body size, resource availability, temperature, salinity, photoperiod, and brood aeration during embryogenesis (Jivoff et al. 2007; Bembe et al. 2017). This suggests that, despite having the same genetic background, embryos can modulate their growth and metabolism in response to environmental temperature (Giménez 2023). However, care must be taken when extrapolating experimental results beyond the studied population, as the relatively small sample size used in this study (n = 6 ovigerous females) imposes limitations on the statistical power of our analyses and the generalizability of the results.

These findings suggest that moderate increases in seawater temperature, such as a rise of 2 °C, could enhance the invasiveness potential of *C. sapidus* by accelerating development, but compromising larval fitness, while extreme temperatures could impose physiological constraints potentially reducing recruitment success (Zheng and Kruse 2000; Orensanz et al. 2004; Wernberg et al. 2013; Marochi et al. 2021). Consequently, this may lead either to the limiting of the local persistence of *C. sapidus* population or to changes in the geographical distribution north of its current range, as this species has evolved specific behaviours to adapt to colder waters in the native area (Johnson 2015; Glandon et al. 2019; Crane et al. 2024). However, results in this study are of limited application to acknowledge this expansion, so further research on exploring lower thermal limits (below 21 °C) may help to assess the overwintering survivorship between European populations, as in Molina et al. (2021).

This study aims to compare our results with embryos from other crustacean species, especially with the native ones (e.g. the European green crab, *Carcinus maenas*). This could offer key findings in order to explore species-specific responses and ecological implications of thermal stress on early developmental stages, in a climate warming context.

Conclusion

This study provides experimental evidence that increasing temperatures above 24 °C constrain embryo performance in *Callinectes sapidus*, demonstrating an optimal thermal window between 22 °C and 24 °C for successful embryogenesis.

The study shows that ETS activity increases and fluctuates at temperatures above 22–24 °C, indicating metabolic stress and a narrowing of aerobic performance capacity in *C. sapidus* embryos. This is supported by reduced egg size and early hatching of abnormal larvae. Elevated temperatures speed up development and may aid invasiveness but also reduce larval fitness and survival, affecting population recruitment. Maternal effects contribute to developmental variability, although genetics alone do not explain all the differences. Future research should include direct oxygen consumption measurements and stress biomarkers to refine our understanding of thermal limits during early developmental stages.

Given projected seawater warming by 2100, these insights are crucial for predicting species resilience and future population dynamics. While this study tested temperatures up to 26 °C, previous research on *Carcinus maenas* larvae has focused on temperatures up to 24 °C, showing variable responses among popu-

lations that may reflect local adaptations (Šargač et al. 2022). Further research should extend thermal gradients beyond 26 °C and compare responses with native crustaceans (e.g. *C. maenas*) to better evaluate competitive interactions and species resilience under future climate scenarios.

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Additional information

Conflict of interest

The authors have declared that no competing interests exist.

Ethical statement

No ethical statement was reported.

Use of AI

No use of AI was reported.

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Data availability

The data underpinning the analysis reported in this paper are deposited in the Zenodo Data Repository at https://zenodo.org/records/15658894.

References

Amsler MO, George RY (1984) Seasonal variation in the biochemical composition of the embryos of *Callinectes sapidus*, Rathbun. Journal of Crustacean Biology 4: 546–553. https://doi.org/10.2307/1548068

Anger K (2006) Contributions of larval biology to crustacean research: A review. Invertebrate Reproduction & Development 49: 175–205. https://doi.org/10.1080/07924259.2006.9652207

Bembe S, Liang D, Chung JS (2017) Optimal temperature and photoperiod for the spawning of blue crab, *Callinectes sapidus*, in captivity. Aquaculture Research 48: 5498–5505. https://doi.org/10.1111/are.13366

Brante A, Fernández M, Eckerle L, Mark F, Pörtner HO, Arntz W (2003) Reproductive investment in the crab *Cancer setosus* along a latitudinal cline: Egg production, embryo losses and embryo ventilation. Marine Ecology Progress Series 251: 221–232. https://doi.org/10.3354/meps251221

Costlow JD (1967) The effect of salinity and temperature on survival and metamorphosis of megalops of the blue crab *Callinectes sapidus.* Helgoländer Wissenschaftliche Meeresuntersuchungen 15: 84–97. https://doi.org/10.1007/BF01618611

Costlow J, Bookhout C (1959) The larval development of *Callinectes sapidus*, Rathbun reared in the laboratory. The Biological Bulletin 116: 373–396. https://doi.org/10.2307/1538947

Crane LC, Burke EA, Gutzler BC, Goldstein JS (2024) Evidence of a Blue Crab (*Callinectes sapidus*) Successfully Overwintering in a Southern Maine Salt Marsh. Northeastern Naturalist 31. https://doi.org/10.1656/045.031.0307

Daly BJ, Eckert GL, Long WC (2021) Moulding the ideal crab: Implications of phenotypic plasticity for crustacean stock enhancement. ICES Journal of Marine Science 78: 421–434. https://doi.org/10.1093/icesjms/fsaa043

Davis CC (1965) A study of the hatching process in aquatic invertebrates: XX. The blue crab, *Callinectes sapidus*, Rathbun, XXI. The nemertean, *Carcinonemertes carcinophila* (Kölliker). Chesapeake Science 6: 201–208. https://doi.org/10.2307/1350814

Epifanio CE (2019) Early life history of the blue crab *Callinectes sapidus*: A review. Journal of Shellfish Research 38: 1–22. https://doi.org/10.2983/035.038.0101

Foxon GEH (1934) Notes on the Swimming Methods and Habits of Certain Crustacean Larvæ. Journal of the Marine Biological Association of the United Kingdom 19: 829–849. https://doi.org/10.1017/S0025315400046816

Gaudencio M, Guerra M (1979) Note on the blue crab *Callinectes sapidus* Rathbun 1896 (Crustacea Decapoda Brachyura) capture in the Tagus estuary. Boletim do Instituto Nacional de investigacao das Pescas 2: 67–73.

Giménez L (2023) A geometric approach to understanding biological responses to environmental fluctuations from the perspective of marine organisms. Marine Ecology Progress Series 721: 17–38. https://doi.org/10.3354/meps14414

Giménez L, Exton M, Spitzner F, Meth R, Ecker U, Jungblut S, Harzsch S, Saborowski R, Torres G (2020) Exploring larval phenology as predictor for range expansion in an invasive species. Ecography 43: 1423–1434. https://doi.org/10.1111/ecog.04725

Glandon HL, Halimeda Kilbourne K, Miller TJ (2019) Winter is (not) coming: Warming temperatures will affect the overwinter behavior and survival of blue crab. PLoS ONE 14: 1–13. https://doi.org/10.1371/journal.pone.0219555

González-Ortegón E, Moreno-Andrés J (2021) Anthropogenic modifications to estuaries facilitate the invasion of non-native species. Processes (Basel, Switzerland) 9: 1–9. https://doi.org/10.3390/pr9050740

González-Ortegón E, Laiz I, Sánchez-Quiles D, Cobelo-Garcia A, Tovar-Sánchez A (2019) Trace metal characterization and fluxes from the Guadiana, Tinto-Odiel and Guadalquivir estuaries to the Gulf of Cadiz. The Science of the Total Environment 650: 2454–2466. https://doi.org/10.1016/j.scitotenv.2018.09.290

González-Ortegón E, Jenkins S, Galil BS, Drake P, Cuesta JA (2020) Accelerated invasion of decapod crustaceans in the southernmost point of the Atlantic coast of Europe: A non-natives' hot spot? Biological Invasions 22: 3487–3492. https://doi.org/10.1007/s10530-020-02345-y

González-Ortegón E, Berger S, Encarnação J, Chairi H, Morais P, Teodósio MA, Oliva-Paterna FJ, Schubart CD, Cuesta JA (2022) Free pass through the pillars of Hercules? Genetic and historical insights into the recent expansion of the atlantic blue crab *Callinectes sapidus* to the west and the east of the Strait of Gibraltar. Frontiers in Marine Science 9: 1–6. https://doi.org/10.3389/fmars.2022.918026

Graham DJ, Perry H, Biesiot P, Fulford R (2012) Fecundity and egg diameter of primiparous and multiparous blue crab *Callinectes sapidus* (Brachyura: Portunidae) in Mississippi waters. Journal of Crustacean Biology 32: 49–56. https://doi.org/10.1163/193724011X615325

Herrera I, López-Cancio J, Yebra L, Hernández-Léon S (2017) The effect of a strong warm winter on subtropical zooplankton biomass and metabolism. Journal of Marine Research 75: 557–577. https://doi.org/10.1357/002224017822109523

Herrera I, Yebra L, Antezana T, Giraldo A, Färber-Lorda J, Hernández-León S (2019) Vertical variability of *Euphausia distinguenda* metabolic rates during diel migration into the oxygen minimum zone of the Eastern Tropical Pacific off Mexico. Journal of Plankton Research 41: 165–176. https://doi.org/10.1093/plankt/fbz004

Herrera I, de Carvalho-Souza GF, González-Ortegón E (2024) Physiological responses of the invasive blue crabs *Callinectes sapidus* to salinity variations: Implications for adaptability and invasive success. Comparative Biochemistry and Physiology. Part A, Molecular & Integrative Physiology 297: 111709. https://doi.org/10.1016/j.cbpa.2024.111709

Hill J, Fowler DL, Van Den Avyle MJ (1989) Species profiles: Life histories and environmental requirements of coastal fishes and invertebrates (Mid-Atlantic) - blue crab. U.S. Fish and Wildlife Service Biological Report 82: 18. https://apps.dtic.mil/sti/citations/ADA210181

Jacobs JR, Biesiot PM, Perry HM, Trigg C (2003) Biochemical composition of embryonic blue crabs *Callinectes sapidus* Rathbun 1896 (Crustacea: Decapoda) from the Gulf of Mexico. Bulletin of Marine Science 72: 311–324. https://aquila.usm.edu/fac_pubs/8676

Jivoff P, Hines AH, Quackenbush LS (2007) Reproduction biology and embryonic development. The Blue Crab: *Callinectes sapidus*, 255–298.

Johnson DS (2015) The savory swimmer swims north: A northern range extension of the blue crab *Callinectes sapidus?* Journal of Crustacean Biology 35: 105–110. https://doi.org/10.1163/1937240X-00002293

Kobayashi S, Matsuura S (1995) Egg development and variation of egg size in the Japanese mitten crab *Eriocheir japonicus* (De Haan). Benthos Research 1995: 29–39. https://doi.org/10.5179/benthos1990.1995.48_29

Marochi MZ, Costa TM, Buckley LB (2021) Ocean warming is projected to speed development and decrease survival of crab larvae. Estuarine, Coastal and Shelf Science 259. https://doi.org/10.1016/j.ecss.2021.107478

Molina AI, Cerrato RM, Nye JA (2021) Population level differences in overwintering survivorship of blue crabs (*Callinectes sapidus*): A caution on extrapolating climate sensitivities along latitudinal gradients. PLoS ONE 16: 1–23. https://doi.org/10.1371/journal.pone.0257569

Morais P, Gaspar M, Garel E, Baptista V, Cruz J, Cerveira I, Leitão F, Teodósio MA (2019) The Atlantic blue crab *Callinectes sapidus* Rathbun, 1896 expands its non-native distribution into the Ria Formosa lagoon and the Guadiana estuary (SW-Iberian Peninsula, Europe). BioInvasions Records 8: 123–133. https://doi.org/10.3391/BIR.2019.8.1.14

Nehring S (2011) Invasion history and success of the American blue crab *Callinectes sapidus* in European and adjacent waters. In: Galil B, Clark P, Carlton J (Eds) In the wrong place - alien marine crustaceans: Distribution, biology and impacts. Invading Nature. Springer Series in Invasion Ecology, Springer, Dordrecht, 607–624. https://doi.org/10.1007/978-94-007-0591-3

Olmi EJ, Orth RJ (1995) Introduction to the proceedings of the blue crab recruitment symposium. Bulletin of Marine Science 57: 707–712. https://scholarworks.wm.edu/vimsarticles/1538

Orensanz JL, Ernst B, Armstrong DA, Stabeno PJ, Livingston P (2004) Contraction of the geographic range of distribution of snow crab (*Chionoecetes opilio*) in the eastern Bering Sea: An environmental ratchet? CalCOFI Reports 45: 65–79. http://www.calcofi.org/publications/calcofireports/v45/CalCOFI_Rpt_Vol_45_2004.pdf

Owens TG, King FD (1975) The Measurement of Respiratory Electron-Transport-System Activity in Marine Zooplankton. Marine Biology 30: 27–36. https://doi.org/10.1007/BF00393750

Packard TT (1971) The measurement of respiratory electron transport activity in marine phytoplankton. Journal of Marine Research 29: 234–244. https://elischolar.library.yale.edu/journal_of_marine_research/1216

Pörtner HO, Bock C, Mark FC (2017) Oxygen- & capacity-limited thermal tolerance: Bridging ecology & physiology. The Journal of Experimental Biology 220: 2685–2696. https://doi.org/10.1242/jeb.134585

Pörtner H-O, Roberts DC, Masson-Delmotte V, Zhai P, Tignor M, Poloczanska E, Mintenbeck K, Alegría A, Nicolai M, Okem A, Petzold J, Rama B (Eds.) NMW (2019) IPCC Special Report on the Ocean and Cryosphere in a Changing Climate IPCC, 2019: Summary for Policymakers. https://doi.org/10.1017/CBO9781139177245.003

Prieto L, Navarro G, Rodríguez-Gálvez S, Huertas IE, Naranjo JM, Ruiz J (2009) Oceanographic and meteorological forcing of the pelagic ecosystem on the Gulf of Cadiz shelf (SW Iberian Peninsula). Continental Shelf Research 29: 2122–2137. https://doi.org/10.1016/j.csr.2009.08.007

Rosenberg R, Costlow JD (1976) Synergistic effects of cadmium and salinity combined with constant and cycling temperatures on the larval development of two estuarine crab species. Marine Biology 38: 291–303. https://doi.org/10.1007/BF00391369

Ruiz-Delgado MC, González-Ortegón E, Herrera I, Drake P, Almón B, Vilas C, Baldó F (2019) Physiological responses to estuarine stress gradient affect performance and field distribution of the non-native crustacean *Synidotea laticauda.* Estuarine, Coastal and Shelf Science 225: 106233. https://doi.org/10.1016/j.ecss.2019.05.015

Rumrill SS (1990) Natural mortality of marine invertebrate larvae. Ophelia 32: 163–198. https://doi.org/10.1080/00785236.1990.10422030

Sánchez-Leal RF, Bellanco MJ, Fernández-Salas LM, García-Lafuente J, Gasser-Rubinat M, González-Pola C, Hernández-Molina FJ, Pelegrí JL, Peliz A, Relvas P, Roque D, Ruiz-Villarreal M, Sammartino S, Sánchez-Garrido JC (2017) The Mediterranean Overflow in the Gulf of Cadiz: A rugged journey. Science Advances 3: 1–11. https://doi.org/10.1126/sciadv.aao0609

Sandifer PA (1975) The role of pelagic larvae in recruitment to populations of adult decapod crustaceans in the York River Estuary and adjacent lower Chesapeake Bay, Virginia. Estuarine and Coastal Marine Science 3: 269–279. https://doi.org/10.1016/0302-3524(75)90028-6

Šargač Z, Giménez L, González-Ortegón E, Harzsch S, Tremblay N, Torres G (2022) Quantifying the portfolio of larval responses to salinity and temperature in a coastal-marine invertebrate: A cross population study along the European coast. Marine Biology 169: 1–18. https://doi.org/10.1007/s00227-022-04062-7

Simčič T, Brancelj A (2004) Respiratory electron transport system (ETS) activity as an estimator of the thermal tolerance of two *Daphnia* hybrids. Journal of Plankton Research 26: 525–534. https://doi.org/10.1093/plankt/fbh056

Simčič T, Pajk F, Jaklič M, Brancelj A, Vrezec A (2014) The thermal tolerance of crayfish could be estimated from respiratory electron transport system activity. Journal of Thermal Biology 41: 21–30. https://doi.org/10.1016/j.jtherbio.2013.06.003

Smith PK, Krohn RI, Hermanson GT, Mallia AK, Gartner FH, Provenzano MD, Fujimoto EK, Goeke NM, Olson BJ, Klenk DC (1985) Measurement of protein using bicinchoninic acid. Analytical Biochemistry 150: 76–85. https://doi.org/10.1016/0003-2697(85)90442-7

Squires HJ (1990) Decapod crustacea of the Atlantic coast of Canada. Canadian Bulletin of Fisheries and Aquatic Sciences: 532.

Styf HK, Nilsson Sköld H, Eriksson SP (2013) Embryonic response to long-term exposure of the marine crustacean *Nephrops norvegicus* to ocean acidification and elevated temperature. Ecology and Evolution 3: 5055–5065. https://doi.org/10.1002/ece3.860

Sulkin S (1984) Behavioral basis of depth regulation in the larvae of brachyuran crabs. Marine Ecology Progress Series 15: 181–205. https://doi.org/10.3354/meps015181

Sulkin SD, Phillipse I, Van Heukelem W (2000) On the Locomotory Rhythm of Brachyuran Crab Larvae and Its Significance in Vertical Migration. Marine Ecology Progress Series 1: 331–335. https://doi.org/10.3354/meps001331

Taylor DL, Fehon MM, Cribari KJ, Scro AK (2022) Blue crab *Callinectes sapidus* dietary habits and pre dation on juvenile winter flounder *Pseudopleuronectes americanus* in southern New England tidal rivers. Marine Ecology Progress Series 681: 145–167. https://doi.org/10.3354/meps13909

Wernberg T, Smale DA, Tuya F, Thomsen MS, Langlois TJ, De Bettignies T, Bennett S, Rousseaux CS (2013) An extreme climatic event alters marine ecosystem structure in a global biodiversity hotspot. Nature Climate Change 3: 78–82. https://doi.org/10.1038/nclimate1627

Young CM (1995) Behavior and Locomotion During the Dispersal Phase of Larval Life. In: McEdward LR (Ed.) Ecology of Marine Invertebrate Larvae 29. CRC Press, Boca Raton, 249–277. https://doi.org/10.1201/9780138758950-8

Zheng J, Kruse GH (2000) Recruitment patterns of Alaskan crabs in relation to decadal shifts in climate and physical oceanography. ICES Journal of Marine Science 57: 438–451. https://doi.org/10.1006/jmsc.1999.0521

Zuur AF, Ieno EN, Walker N, Saveliev AA, Smith GM (2009) Smart Society: A Sociological Perspective on Smart Living Mixed Effects Models and Extensions in Ecology with R. Springer, 579 pp. https://doi.org/10.4324/9780429201271-2

Zuur AF, Ieno EN, Elphick CS (2010) A protocol for data exploration to avoid common statistical problems. Methods in Ecology and Evolution 1: 3–14. https://doi.org/10.1111/j.2041-210x.2009.00001.x

Supplementary material 1

Figures and tables of the statistical analysis for both dependent variables (ETS activity on embryos and egg size)

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